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Roles of Catecholamines in the Regulation of Stress-induced Hypothalamo-Pituitary Adrenal(HPA) axis Stimulation

Stress시 시상하부-뇌하수체-부신계 조절에 대한 catecholamine 신경전달물질의 역할에 관한 연구

Yoo Hun Suh, Jung Kyoo Lim, and Chan Woong Park
Department of Pharmacology, College of Medicine, Seoul National University,

It is generally recognized that central nervous system plays an essential role in the regulation of synthesis and release of ACTH. It is now firmly estabilished that the hypothalamus is the focal point at which neural stimuli converge to influence the secretion of ACTH, and the median eminence is regarded as the final common path through which information is transmitted to the anterior pituitary.

In recent years, efforts have been directed toward and progress has been made in identifying some of the neurotransmitters that control the release of corticotropin-releasing factor (CRF). Among them, monoaminergic neural systems in the hypothalmic control of pituitary function have been widely studied.

A possible role of brain catecholamines in the regulation of ACTH secretion has been considered by numerous investigators. The catecholamines are not per se the hypothalamic corticotropin releasing factors (Saffran and Schally, 1955; Guillemin and Rosenberg, 1955; Martini et al., 1960; Fischer and Moriarty, 1977).

Several groups of investigators have reported increased adrenal corticosteroid secretion following the injection of catecholamines directly into the brain (Endrocozi et al., 1963; Krieger and Krieger, 1965).

On the other hand numerous studies have failed to support an excitatory role of brain catecholamines in the regulation of ACTH secretion but have led to the opposite (Smelik, 1967; Carr and Moore, 1968; Van Loon et al., 1971a,b,c; Scapagnini et al., 1970, 1971; Ganong et al., 1976; Ganong, 1977; Buckingham and Hodges, 1977; Steiner and Grahame-Smith, 1980).

This study was undertaken to try to clarify the role of catecholamines (norepinephrine and dopamine) in the hypothalamo-pituitary-adrenocortical response to stress. The aims of the present study were to check the kinetic parameters of turnover of catecholamines as well as steady state concentrations before and after ether stress.

MATERIALS AND METHODS

Animals and Chemicals

In all experiments male Sprague-Dawley rats (SNU animal house, Seoul, Korea) weighing 160~250g were used. Animals were housed five to a cage in a constant-temperature room (20°~25°C) with a 12-hr light cycle (lights on 7.00~19.00 hours) and given commercial ratchow

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and tap water ad libitum. Animals were allowed to acclimatize to the condition of a quiet laboratory for 1 hr before experimental procedures were started. Great care was taken to minimize the disturbance to the animals during the transfer. Experiments were performed between 10 h and 12 h.

Stress

Stress was induced by exposing the rat for 1 min to an atmosphere saturated in ether vapor at room temperature (20~24°C).

Determination of plasma corticosterone levels

Rats were killed by decapitation and blood was collected from the blood vessels of the severed neck into heparinized tubes. Care was taken to ensure that the rats following in line did not view the decapitation of the preceding animals.

The plasma corticosterone was estimated by the spectrofluorimetric method of Zenker and Bernstein(1958). Fluorimetric readings were made on Perkin-Elmer Spectrophotofluorimeter (Model 1000, England) at an excitation wavelength of 468nm and an emission wavelength of 520nm. Corticosterone standard was obtained from the Sigma Chemical Company Ltd.

Determination of Contents of Norepinephrine and Dopamine in Rat Brain

Brain fractions were weighed and homogenized in 10ml of acid butanol containing sodium sulfite and EDTA. The postcentrifugation supernatant was shaken in the presence of, water, sodium acetate, and 200ng activated alumina as described by Ansell and Beeson (1968). The alumina was washed and adsorbed, catecholamines were eluted with 0.5M phosphate buffer, pH 6.5. The quantity of norepinephrine (NE) and dopamine (DA) were determined with a fluorimetric assay described by Ansell and Beeson(1968).

Determination of turnover rates of norepinephrine and dopamine in whole brain.

Catecholamine turnover rates were determined by inhibiting synthesis by intraperitoneally administering α-methyl-para-tyrosine (α-MT, Sigma) at a dose of 250 mg/kg. Rats were sacrificed by decapitation at defined time intervals(0, 1, 2, and 4 hours) after injection of α-MT and the brains were immediately removed and frozen on dry ice and stored at −25°C until used. On each time interval, 6 to 8 animals per group were used. The synthesis rates were calculated based on the measurement of both the steady state levels of catecholamines and the rate constant of amine loss in rat brain. The rate constant was determined from the slope of the exponential decline of amines as described by Brodie et al.(1966).

RESULTS

1. Time course of the elevation of plasma corticosterone, norepinephrine (NE) and dopamine(DA) levels in rat brain after 1 min-ether stress

Steady state brain NE and DA concentrations in the hypothalamus and in the remainder of brain after 1 min-ether stress were immediately and significantly elevated without significant rise in the levels of plasma corticosterone, but the levels of plasma corticosterone were significantly increased after 2 minutes and reached a maximum at 10 min after stress, when NE and DA reached minimum levels(Fig. 1 and Fig. 2).

2. Kinetic parameters of NE and DA turnover in rat whole brain before and after stress (1 min-ether stress)

Figure 3 and 4 show the time-course of the disappearance of brain NE and DA stores in rats after the administrations of tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine(250mg/

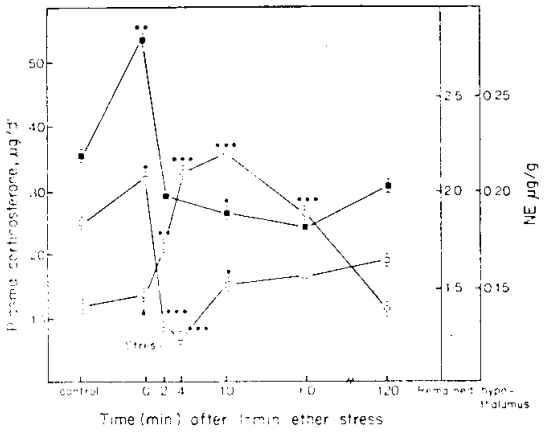

Fig. 1. Time course of the elevation of plasma corticosterone(○) and NE levels in rat hypothalamus(□) and remainder brain areas(■) after 1 min.-ether stress.
Mean values±S.E.M. for 6 to 14 rats per group were shown.
S.E.M. were indicated only when they exceed the size of symbol.
Asterisks indicate significant differences from the Non-stressed control group (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

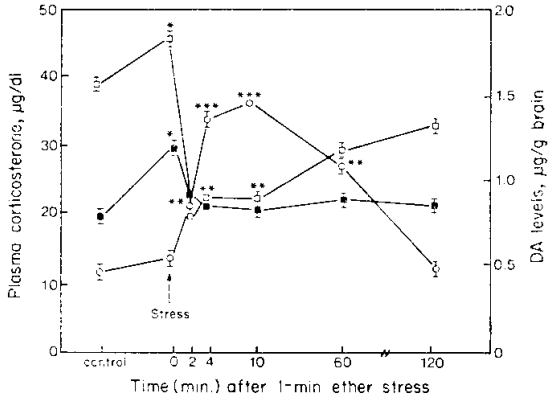

Fig. 2. Time course of the elevation of plasma corticosterone(○) and DA levels in rat hypothalamus(□) and remainder brain areas(■) after 1 min-ether stress.
Mean values±S.E.M. for 6 to 12 rats per group were shown.
S.E.M. were indicated only when they exceed the size of symbol.
Asterisks indicate significant differences from the Non-stressed control group (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

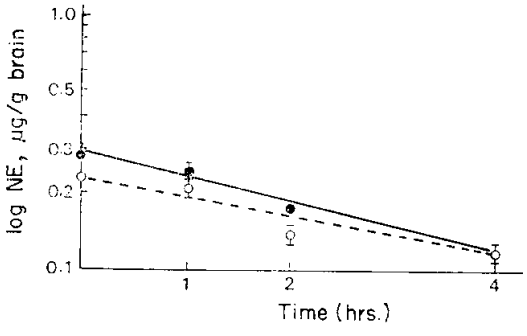

Fig. 3. Disappearance kinetics of NE in rat whole brain after α-methyl-p-tyrosine. Slopes were best fitted by least squares linear regression analysis ($r>0.90$).
Bars represent mean values of at least 6 to 8 animals±S.E.
S.E. were indicated only when they exceed the size of symbol.
Non-stressed control group: ○—○.
1 min-ether stressed group: ●—●.

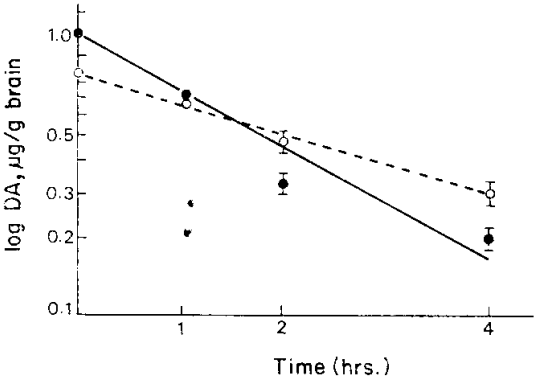

Fig. 4. Disappearance kinetics of DA in rat whole brain after α-methyl-p-tyrosine. Slopes were best fitted by least squares linear regression analysis ($r>0.90$).
Bars represent mean values of at least 6 to 8 animals±S.E.
S.E. were indicated only when they exceed the size of symbol.
Non-stressed control group: ○—○.
1 min-ether stressed group: ●—●.

Table 1. Kinetic parameters of NE turnover in rat whole brain before and after stress

Treatment	Steady state level NE ($\mu g/g \pm$S.E.)[a]	Rate constant of NE turnover ($hr^{-1} \pm$S.E.)[b]	NE turnover time(hr±S.E.)[c]	Synthesis rate of NE ($\mu g/g/hr \pm$S.E.)[d]
Control	0.226±0.076	0.448±0.062	2.221±0.213	0.101±0.030
Ether stress	0.287±0.054* (+27.0%)	0.543±0.081** (+21.2%)	1.840±0.102** (−17.1%)	0.156±0.025*** (+54.3%)

[a]The values represent mean±S.E.M. of at least 6 to 8 animals of 2 different experiments.
[b,c,d]The values represent mean±S.E.M. of 2 different experiments and measured from the decline of DA after α-methyl paratyrosine (250mg/kg).
( ) : % of increase over control.
* $p<0.05$ compared to control.
** $p<0.01$ compared to control.
*** $p<0.001$ compared to control.

Table 2. Kinetic parameters of DA turnover in rat whole brain before and after stress

Treatment	Steady state level DA ($\mu g/g \pm$S.E.)[a]	Rate constant of DA turnover ($hr^{-1} \pm$S.E.)[b]	DA turnover time (hr±S.E.)[c]	Synthesis rate of DA ($\mu g/g/hr \pm$S.E.)[d]
Control	0.829±0.080	0.636±0.083	1.572±0.110	0.527±0.048
Ether stress	1.230±0.075*** (+48.4%)	1.049±0.102*** (+65.0%)	0.953±0.075*** (−39.4%)	1.290±0.102*** (+144.8%)

[a]The values represent mean±S.E.M. of at least 6 to 8 animals of 2 different experiments.
[b,c,d]The values represent mean±S.E.M. of 2 different experiments and measured from the decline of DA after α-methyl paratyrosine (250mg/kg)
( ) : % of increase over control.
* $p<0.05$ compared to control.
** $p<0.01$ compared to control.
*** $p<0.001$ compared to control.

kg). The slope of NE disappearance during 1 min-ether stress was shown to be slightly steeper compared to that in non-stressed group (Fig. 3). But the slope of DA disappearance during 1 min-ether stress was much steeper than that in non-stressed control group.

As shown in Table 1 and 2, steady state levels and rate constants of NE and DA turnover during 1 min-stress were significantly increased but turnover times of NE and DA were significantly shortened. Also the turnover rates of NE and DA in whole brain during 1 min-ether stress were increased 21%, 65% over control, respectively.

DISCUSSION

The origins of the neurons which exert pharmacological effects on the CRF neurons are at present not defined although tracts inhibiting and stimulating ACTH release have been found in the spinal cord and brain stem (Gann et al., 1978). The secretion of CRF is reflected by compound B secretion into plasma. Corticosterone forms the major component of fluorescent corticoids in the rat.

Rats have a diurnal plasma corticosterone cycle with a trough early in the morning and a peak in the late afternoon (Critchlow, 1963; Lee et al., 1982). Our previous studies demonstrated that the levels of 5-hydroxytryptamine (serotonin) showed definite circadian rhythmicity but the catecholamines didn't (Lee et al., 1982). This has been interpreted as indicating that the circadian rhythmicity of plasma cortico-

sterone may not be more closely related with catecholamines than with 5-HT.

Jones et al. (1976) and Buckingham and Hodges (1977) have demonstrated in the isolated rat hypothalamo-pituitary unit in vitro that acetylcholine and serotonin release CRF and that noradrenaline inhibits this release. While in vitro noradrenaline clearly inhibits CRF release, in the intact animal the situation is more complex. Possible explanations for the conflicting results are that steady state amine concentrations do not provide adequate neuronal activity and that some drugs can affect several neurotransmitter systems and whole body. The aim of these studies was to check the kinetic parameters of turnover of catecholamines as well as steady state concentrations before and after stress.

The present study has demonstrated that steady state concentrations of norepinephrine and dopamine after 1 min-ether stress immediately and significantly rised without significant rise in the levels of plasma corticosterone which increased 2 minutes after stress with peak level at 10 min. Thus, the present findings suggest that the increase in the noradrenergic and dopaminergic neuronal activity precede that in the stress-responsiveness of HPA axis. Our previous results also showed that serotonergic neuronal activity preceded HPA activity to stress (Suh et al., 1983).

But the contents of NE and DA reached the minimum levels between 4 and 10 minutes, when the plasma concentrations of corticosterone reached a maximum. This may indicate that NE and DA may inhibit the stress responsiveness of HPA axis.

It has been shown that turnover rates and rate constants of norepinephrine and dopamine in whole brain in the stressed group were moderately greater than that in the non-stressed control group. This suggests that stress causes increases in synthesis and turnover of norepinephrine and dopamine and that the functional catecholamine responses to stress precede the rise in plasma corticosterone.

Previously, we reported that the turnover rates of 5-HT in rat brain were markedly increased during ether stress (Suh et al., 1983).

In conclusion, the present study strongly suggests that the turnover or synthesis rates of catecholamines of rat whole brain are moderately increased in response to stress and that neuronal activities of catecholamines precede the HPA activity to stress.

SUMMARY

A possible role of brain catecholamines in the regulation of HPA axis has been considered by numerous investigators.

The present study was undertaken to check the kinetic parameters of turnover of catecholamines (NE and DA) as well as steady state concentrations before and after ether stress.

Steady state brain NE and DA concentrations in the rat hypothalamus and in the remainder of the brain after 1 min-ether stress were immediately and significantly elevated without rise in the levels of plasma corticosterone, but the levels of plasma corticosterone were significantly increased after 2 minutes and reached a maximum at 10 min after stress, when NE and DA reached minimum levels.

The turnover rates and rate constants of NE and DA turnover in rat whole brain during 1 min-stress were significantly increased.

In conclusion, the present study strongly suggests that the synthesis or turnover rates of catecholamines of rat whole brain are moderately increased in response to ether stress and that neuronal activities of catecholamines precede the HPA activity to stress.

=국문초록=

Stess시 시상하부-뇌하수체-부신계 조절에 대한 신경전달물질의 역할에 관한 연구

서울대학교 의과대학 약리학교실
서유헌 · 임정규 · 박찬웅

오래전부터 stress시 corticotropin-releasing factor (CRF) 분비를 조절하는데 catecholamine이 관여한다는 사실이 알려져 왔지만 정확한 조절기능에 대해서는 많은 이견을 보이고 있다. 따라서 저자들은 catecholamine (norepinephrine, dopamine)이 stress시 HPA axis를 조절하는데 어떤 역할을 하는가를 알아보기 위해서 stress 전후에 norepinephrine(NE), dopamine(DA)의 steady state 농도와 합성율(synthesis rate) 및 교체율(turnover rate)을 측정분석해 보았다.

1분간 ether stress를 가한 직후에 시상하부와 다른 전뇌 부위에서의 NE과 DA 농도는 주각적으로 의미있는 상승을 보였으나 혈장 corticosterone 농도는 2분후부터 상승을 보였다. 또한 corticosterone농도는 10분후에 최고치에 도달하였으며 이때 NE과 DA는 최소치를 보였다.

NE와 DA의 합성율 및 교체율은 1분간 ether stress 가할 시에 의미있게 상승하였으며 교체시간(turnover time)은 의미있는 감소를 보였다.

이상의 결과를 종합해 볼 때 catecholamine의 전뇌에서의 합성율 및 교체율은 스트레스시 증가하며, catecholamine 신경세포 활동이 시상하부-뇌하수체-부신계의 스트레스 반응을 선행해서 조절하고 있다고 생각된다.

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